

STIC-ILL

284480

From: Davis, Minh-Tam
Sent: Friday, February 25, 2000 3:46 PM
To: STIC-ILL
Subject: Reprint request

For 09/047652.amend

1) Weizman, R, 1993, Clinical neuropharmacology, 16(5): 401-417

2) Casalotti SO, 1992, Gene 121(2): 377-382.

Thank you.

Minh-Tam Davis

ART UNIT 1642, Room 8A01

305-2008

ZGE-0002280432

N. N^o 2/25

antagonist
no Ab to PBR

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Set  Items  Description
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? s pbr or peripheral (w) type (w) benzodiazepine (5n) receptor

      1234  PBR
      604261 PERIPHERAL
      1867298 TYPE
      44840  BENZODIAZEPINE
      1135761 RECEPTOR
      477   PERIPHERAL (W) TYPE (W) BENZODIAZEPINE (5N) RECEPTOR
S1     1559  PBR OR PERIPHERAL (W) TYPE (W) BENZODIAZEPINE (5N) RECEPTOR
? s antibod?
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S2 1134322  ANTIBOD?
? s s1 and s2
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      1559  S1
      1134322 S2
S3      81  S1 AND S2
? s antagonist or reduc? or inhibit?
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Processing

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      242543  ANTAGONIST
      2389041  REDUC?
      2024179  INHIBIT?
S4 4095005  ANTAGONIST OR REDUC? OR INHIBIT?
? s s3 and s4
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      81  S3
      4095005 S4
S5      27  S3 AND S4
? rd
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>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records

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S6      19  RD (unique items)
? t s6/3,k,ab/1-19
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6/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09849770 99151530

Peripheral-type benzodiazepine receptor (
PBR) in human breast cancer: correlation of breast cancer cell
aggressive phenotype with **PBR** expression, nuclear localization, and
PBR-mediated cell proliferation and nuclear transport of cholesterol.
Hardwick M; Fertikh D; Culty M; Li H; Vidic B; Papadopoulos V
Department of Cell Biology, Georgetown University Medical Center,
Washington, DC 20007, USA.
Cancer Res (UNITED STATES) Feb 15 1999, 59 (4) p831-42, ISSN
0008-5472 Journal Code: CNF
Contract/Grant No.: ES-07747, ES, NIEHS; P50-CA-58185, CA, NCI; HD-01031,
HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE.

Aberrant cell proliferation and increased invasive and metastatic behavior are hallmarks of the advancement of breast cancer. Numerous studies implicate a role for cholesterol in the mechanisms underlying cell proliferation and cancer progression. The **peripheral-type benzodiazepine receptor (PBR)** is an Mr 18,000 protein primarily localized to the mitochondria. PBR mediates cholesterol transport across the mitochondrial membranes in steroidogenic cells. A role for PBR in the regulation of tumor cell proliferation has also been shown. In this study, we examined the expression, characteristics, localization, and function of PBR in a battery of human breast cancer cell lines differing in their invasive and chemotactic potential as well as in several human tissue biopsies. Expression of PBR ligand binding and mRNA was dramatically increased in the highly aggressive cell lines, such as MDA-231, relative to nonaggressive cell lines, such as MCF-7. PBR was also found to be expressed at high levels in aggressive metastatic human breast tumor biopsies compared with normal breast tissues. Subcellular localization with both **antibodies** and a fluorescent PBR drug ligand revealed that PBR from the MDA-231 cell line as well as from aggressive metastatic human breast tumor biopsies localized primarily in and around the nucleus. This localization is in direct contrast to the largely cytoplasmic localization seen in MCF-7 cells, normal breast tissue, and to the typical mitochondrial localization seen in mouse tumor Leydig cells. Pharmacological characterization of the receptor and partial nucleotide sequencing of PBR cDNA revealed that the MDA-231 PBR is similar, although not identical, to previously described PBR. Addition of high affinity PBR drug ligands to MDA-231 cells increased the incorporation of bromodeoxyuridine into the cells in a dose-dependent manner, suggesting a role for PBR in the regulation of MDA-231 cell proliferation. Cholesterol uptake into isolated MDA-231 nuclei was found to be 30% greater than into MCF-7 nuclei. High-affinity PBR drug ligands regulated the levels of cholesterol present in MDA-231 nuclei but not in MCF-7. In addition, the PBR-dependent MDA-231 cell proliferation was found to highly correlate ($r = -0.99$) with the PBR-mediated changes in nuclear membrane cholesterol levels. In conclusion, these data suggest that PBR expression, nuclear localization, and PBR-mediated cholesterol transport into the nucleus are involved in human breast cancer cell proliferation and aggressive phenotype expression, thus participating in the advancement of the disease.

Peripheral-type benzodiazepine receptor (PBR) in human breast cancer: correlation of breast cancer cell aggressive phenotype with PBR expression, nuclear localization, and PBR-mediated cell proliferation and nuclear transport of cholesterol. ... implicate a role for cholesterol in the mechanisms underlying cell proliferation and cancer progression. The **peripheral-type benzodiazepine receptor (PBR)** is an Mr 18,000 protein primarily localized to the mitochondria. PBR mediates cholesterol transport across the mitochondrial membranes in steroidogenic cells. A role for PBR in the regulation of tumor cell proliferation has also been shown. In this study, we examined the expression, characteristics, localization, and function of PBR in a battery of human breast cancer cell lines differing in their invasive and chemotactic potential as well as in several human tissue biopsies. Expression of PBR ligand binding and mRNA was dramatically increased in the highly aggressive cell lines, such as MDA-231, relative to nonaggressive cell lines, such as MCF-7. PBR was also found to be expressed at high levels in aggressive metastatic human breast tumor biopsies compared with normal breast tissues. Subcellular localization with both **antibodies** and a fluorescent PBR drug ligand revealed that PBR from the MDA-231 cell line as well as from aggressive metastatic human breast tumor...

... in mouse tumor Leydig cells. Pharmacological characterization of the receptor and partial nucleotide sequencing of PBR cDNA revealed that

5/3,K,AB/30 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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2/15/00

02734098 Genuine Article#: LZ577 Number of References: 123
Title: MOLECULAR CELLULAR AND BEHAVIORAL-ASPECTS OF PERIPHERAL-TYPE
BENZODIAZEPINE RECEPTORS

✓

Author(s): WEIZMAN R; GAVISH M

Corporate Source: TEL AVIV COMMUNITY MENTAL HLTH CTR, 9 HATZVI ST, RAMAT
HATAYASSIM/IL-67197 TEL AVIV//ISRAEL/; TEL AVIV UNIV, SACKLER FAC
MED/IL-69978 TEL AVIV//ISRAEL/; TECHNION ISRAEL INST TECHNOL, BRUCE
RAPPAPORT FAC MED, DEPT PHARMACOL/HAIFA//ISRAEL/

Journal: CLINICAL NEUROPHARMACOLOGY, 1993, V16, N5 (OCT), P401-417

ISSN: 0362-5664

Language: ENGLISH Document Type: REVIEW

Abstract: Peripheral-type benzodiazepine receptors (PBR) are prominent in peripheral organs, whereas in the brain, they are sparse and located mainly in glial cells. The PBR bind with high affinity the ligands Ro 5-4864 (4'-chlorodiazepam) and PK 11 195 (an isoquinoline carboxamide derivative), but not clonazepam, which binds with high affinity to central-type benzodiazepine receptors (CBR). Subcellularly, PBR are predominantly localized on the outer mitochondrial membrane. It appears that the PBR are composed of three subunits: an 18-kDa subunit that binds isoquinoline carboxamide derivatives; a 30-kDa subunit that binds benzodiazepines; and a 32-kDa subunit labeled by the benzodiazepine [H-3]AHN 086, the voltage-dependent anion channel. Recently, complementary DNA (cDNA) encoding for rat and human PBR was isolated and sequenced. The PBR gene is located in the q13.3 region of the long arm of human chromosome 22. The PBR play a major role in steroidogenesis, controlling cholesterol mitochondrial transport. Diazepam-binding inhibitor and its processing products, as well as porphyrins, have been suggested as putative endogenous ligands for these receptors. The PBR ligands have been shown to control cell proliferation and differentiation, and the binding capacity for these ligands is enhanced in some malignant tumors. Stress has been demonstrated to affect PBR bidirectionally. Acute stress is associated with increased PBR density, whereas chronic stress down-regulates PBR.

, 1993

...Abstract: labeled by the benzodiazepine [H-3]AHN 086, the voltage-dependent anion channel. Recently, complementary DNA (cDNA) encoding for rat and human PBR was isolated and sequenced. The

5/3,K,AB/16 (Item 16 from file: 155)
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05815656 90062173

Molecular cloning and expression of cDNA encoding a **peripheral-type benzodiazepine receptor**.

Sprengel R; Werner P; Seeburg PH; Mukhin AG; Santi MR; Grayson DR; Guidotti A; Krueger KE

Laboratory of Molecular Neuroendocrinology, University of Heidelberg, Federal Republic of Germany.

J Biol Chem (UNITED STATES) Dec 5 1989, 264 (34) p20415-21,
ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

This report describes the cloning of a full length cDNA encoding PKBS, a protein of approximately 17 kDa associated with peripheral-type benzodiazepine binding sites. Cyanogen bromide digestion of purified PKBS yielded several peptide fragments which were subjected to gas-phase sequencing. Based on these partial amino acid sequences, oligonucleotide probes were used to screen a rat adrenal cDNA library. Several hybridizing clones were isolated which were found to contain overlapping sequences. The longest cDNA spanned 781 base pairs and specified an open reading frame of 169 amino acids which matched all of the peptide sequences. Northern analysis with this PKBS cDNA probe in different rat tissues revealed one RNA species of approximately 850 nucleotides exhibiting relative abundances qualitatively comparable with the densities of peripheral-type benzodiazepine binding sites in each tissue. The PKBS cDNA was cloned into an eukaryotic expression vector placing it under transcriptional control of the beta-globin promoter and SV40 enhancer. Transfection of the transformed human kidney 293 cell line with this recombinant vector resulted in stoichiometric increases of about 900 fmol/mg of protein in binding capacities for Ro5-4864 (4'-chlorodiazepam) and PK 11195, two peripheral-type benzodiazepine ligands. These increases were accounted for by the expression of binding sites with approximate dissociation constants of 5 nM for PK 11195 and 8 nM for Ro5-4864, thereby distinguishing the expressed binding sites as being characteristic of the receptor from rat origin rather than of the host human-derived cell line. The rank order of potency of different ligands to compete against [3H]Ro5-4864 binding in the transfected cells was PK 11195 greater than Ro5-4864 greater than diazepam greater than protoporphyrin IX greater than clonazepam, consistent with the specificity characteristic of rat peripheral-type benzodiazepine binding sites. These studies suggest that PKBS comprises binding domains for benzodiazepines and isoquinoline carboxamides and hence is apparently responsible for the manifestation of peripheral-type benzodiazepine recognition sites.

Molecular cloning and expression of cDNA encoding a **peripheral-type benzodiazepine receptor**.

Dec 5 1989,

Descriptors: Adrenal Glands--Metabolism--ME; *Cloning, Molecular; *DNA--Genetics--GE; *Receptors, GABA-A--Genetics--GE; Amino Acid Sequence; Base Sequence; Benzodiazepinones--Metabolism--ME; Blotting, Northern; Convulsants--Metabolism--ME; Cyanogen Bromide; Gene Expression; Gene Library; Mitochondria--Metabolism--ME; Molecular Sequence Data; Oligonucleotide Probes; Peptide Fragments--Isolation and Purification--IP...

Chemical Name: Benzodiazepinones; (Convulsants; (Oligonucleotide Probes; (Peptide Fragments; (Receptors, GABA-A; (Ro 5-4864; (Cyanogen Bromide; (

DNA

5/3,K,AB/17 (Item 17 from file: 155)
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05518114 89094317

Effect of benzodiazepines on the proliferation of mouse spleen lymphocytes in vitro.

Pawlikowski M; Lyson K; Kunert-Radek J; Stepien H
Department of Experimental Endocrinology, Medical Academy of Lodz, Poland.

J Neural Transm (AUSTRIA) 1988, 73 (2) p161-6, ISSN 0300-9564

Journal Code: JAJ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The effect of several benzodiazepines (clonazepam, diazepam, Ro 5-4864, Ro 15-1788) and two pineal gland indoleamines (N-acetylserotonin,

/3,K,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06937751 91146565

Molecular cloning and chromosomal localization of a human **peripheral-type benzodiazepine receptor**.

Riond J; Mattei MG; Kaghad M; Dumont X; Guillemot JC; Le Fur G; Caput D; Ferrara P

Laboratoire de Biochimie des Proteines, Sanofi Elf Bio-Recherches, Labege, France.

Eur J Biochem (GERMANY) Jan 30 1991, 195 (2) p305-11, ISSN 0014-2956 Journal Code: EMZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The sequencing of endopeptidase-generated peptides from the peripheral binding site (PBS) for benzodiazepines, purified from a Chinese hamster ovary (CHO) cell line, produced internal sequence information, and confirmed and extended the NH2-terminal PBS sequence that we previously reported. Since the sequences were highly similar to the corresponding rat PBS sequences, we investigated whether they were also conserved in human PBS. Scatchard analysis of [3H]PK11195 (a derivative of isoquinoline carboxamide) binding and photoaffinity labeling with [3H]PK14105 (a nitrophenyl derivative of PK11195) revealed that CHO PBS and human PBS are closely related. Furthermore a rabbit antiserum raised against three peptides synthesized on the basis of the CHO PBS sequence immunoprecipitate the solubilized U937 PBS and also recognize the human protein in an immunoblot analysis. Based on these results, we screened a U937 cell cDNA library with four oligonucleotide probes derived from the CHO sequence. Two of the probes hybridized with several clones that we isolated and sequenced. One of these, h-pPBS11, is 831 nucleotides and contains a full-length representation of human PBS mRNA. The amino acid sequence of human PBS deduced from the cDNA is 79% identical to that reported for rat PBS, however, human PBS contains two cysteines while rat PBS is characterized by the absence of this amino acid. Using the cDNA of human PBS as a probe, the PBS gene was located in the 22q13.3 band of the human genome.

Molecular cloning and chromosomal localization of a human **peripheral-type benzodiazepine receptor**.

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? s peripheral (w) type (5n) benzodiazepine (5n) receptor

599165 PERIPHERAL
1848185 TYPE
44578 BENZODIAZEPINE
1124156 RECEPTOR
S1 536 PERIPHERAL (W) TYPE (5N) BENZODIAZEPINE (5N) RECEPTOR
? s gene or dna

1493948 GENE
1312062 DNA
S2 2255828 GENE OR DNA
? s s1 and s2

536 S1
2255828 S2
S3 96 S1 AND S2
? s s3 and py<=1997

Processing
Processing

96 S3
31227178 PY<=1997
S4 60 S3 AND PY<=1997
? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...examined 50 records (50)
...completed examining records
S5 37 RD (unique items)
? t s5/3,k,ab/1-37

5/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09363579 98070377

Targeted disruption of the **peripheral-type benzodiazepine receptor gene** inhibits steroidogenesis in the R2C Leydig tumor cell line.

Papadopoulos V; Amri H; Li H; Boujrad N; Vidic B; Garnier M
Department of Cell Biology, Georgetown University Medical Center,
Washington, D. C. 20007, USA. papadopv@gunet.georgetown.edu
J Biol Chem (UNITED STATES) Dec 19 1997, 272 (51) p32129-35,
ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: ES-07747, ES, NIEHS; HD-01031, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE

To evaluate the role of the mitochondrial **peripheral-type benzodiazepine receptor** (PBR) in steroidogenesis, we developed a molecular approach based on the disruption of the PBR gene, by homologous recombination, in the constitutive steroid producing R2C rat Leydig tumor cell line. Inactivation of one allele of the PBR gene resulted in the suppression of PBR mRNA and ligand binding expression. Immunoblot and electron microscopic immunogold labeling analyses confirmed

5/3,K,AB/32 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02088191 Genuine Article#: JZ736 Number of References: 17

Title: STRUCTURE OF THE RAT **GENE** ENCODING THE MITOCHONDRIAL
BENZODIAZEPINE RECEPTOR

Author(s): CASALOTTI SO; PELAIA G; YAKOVLEV AG; CSIKOS T; GRAYSON DR;
KRUEGER KE

Corporate Source: GEORGETOWN UNIV, SCH MED, FIDIA GEORGETOWN INST
NEUROSCI, 3900 RESERVOIR RD NW/WASHINGTON//DC/20007; GEORGETOWN UNIV, SCH
MED, FIDIA GEORGETOWN INST NEUROSCI, 3900 RESERVOIR RD
NW/WASHINGTON//DC/20007; GEORGETOWN UNIV, SCH MED, DEPT
PHYSIOL/WASHINGTON//DC/20007

Journal: GENE, 1992, V121, N2 (NOV 16), P377-382

ISSN: 0378-1119

Language: ENGLISH Document Type: NOTE

Abstract: The **gene** encoding the rat mitochondrial benzodiazepine receptor (MBR) was cloned and characterized. Hybridization of a previously cloned cDNA for MBR to genomic Southern blots indicated that the **gene** was probably present at one copy per haploid genome. Rapid amplification of cDNA ends with rat adrenal RNA was used to obtain 47 nt of additional sequence upstream from our previously cloned MBR cDNA proving to be a crucial step in cloning the first exon of this **gene**. The MBR **gene** is comprised of four exons spanning approx. 10 kb. The first intron, contained within a 8-kb stretch of this **gene**, is located within the 5'-untranslated sequence, whereas the remaining two introns are much shorter (641 and 854 bp) and interrupt the coding sequence. The third intron contains sequences homologous to rodent B1 repetitive elements and a novel sequence closely resembling part of a repetitive element belonging to the Alu family in humans. The transcription start point was mapped by S1 nuclease protection assays suggesting that the first exon is just 56 bp in length. The sequence upstream from this region contains three GC boxes but lacks other known consensus recognition sites for sequence-specific transcription factors.

Title: STRUCTURE OF THE RAT **GENE** ENCODING THE MITOCHONDRIAL
BENZODIAZEPINE RECEPTOR

the absence of the 18-kDa PBR protein in the selected clone. Although mitochondria from the PBR-negative cells contained high levels of the constitutively expressed 30-kDa steroidogenic activity regulator protein, these cells produced minimal amounts of steroids compared with normal cells (5%). Moreover, mitochondria from PBR-negative cells failed to produce pregnenolone when supplied with exogenous cholesterol. Addition of the hydrosoluble cholesterol derivative, 22R-hydroxycholesterol, increased steroid production by the PBR-negative R2C cells, indicating that the cholesterol transport mechanism was impaired. Stable transfection of the PBR-negative R2C Leydig cells with a vector containing the PBR cDNA resulted in the recovery of the steroidogenic function of the cells. These data demonstrate that PBR is an indispensable element of the steroidogenic machinery, where it mediates the delivery of the substrate cholesterol to the inner mitochondrial side chain cleavage cytochrome P-450.

Targeted disruption of the **peripheral-type benzodiazepine receptor gene** inhibits steroidogenesis in the R2C Leydig tumor cell line.

Dec 19 1997,

To evaluate the role of the mitochondrial **peripheral-type benzodiazepine receptor** (PBR) in steroidogenesis, we developed a molecular approach based on the disruption of the PBR gene, by homologous recombination, in the constitutive steroid producing R2C rat Leydig tumor cell line. Inactivation of one allele of the PBR gene resulted in the suppression of PBR mRNA and ligand binding expression. Immunoblot and electron microscopic...

; Biological Transport; Cholesterol--Metabolism--ME; DNA , Complementary; Gene Targeting; Leydig Cell Tumor--Pathology--PA; Mitochondria--Metabolism--ME; Mutagenesis; Rats; Transfection; Tumor Cells, Cultured

Chemical Name: DNA, Complementary; (Receptors, GABA-A; (Steroids; (Cholesterol

5/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09178463 97347194

Increased expression of the **peripheral-type benzodiazepine receptor** -isoquinoline carboxamide binding protein mRNA in brain following portacaval anastomosis.

Review

Molecular Cellular and Behavioral Aspects of Peripheral-Type Benzodiazepine Receptors

Ronit Weizman and *Moshe Gavish

*Tel Aviv Community Mental Health Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, and *Department of Pharmacology, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Summary: Peripheral-type benzodiazepine receptors (PBR) are prominent in peripheral organs, whereas in the brain, they are sparse and located mainly in glial cells. The PBR bind with high affinity the ligands Ro 5-4864 (4'-chlorodiazepam) and PK 11195 (an isoquinoline carboxamide derivative), but not clonazepam, which binds with high affinity to central-type benzodiazepine receptors (CBR). Subcellularly, PBR are predominantly localized on the outer mitochondrial membrane. It appears that the PBR are composed of three subunits: an 18-kDa subunit that binds isoquinoline carboxamide derivatives; a 30-kDa subunit that binds benzodiazepines; and a 32-kDa subunit labeled by the benzodiazepine [³H]AHN 086, the voltage-dependent anion channel. Recently, complementary DNA (cDNA) encoding for rat and human PBR was isolated and sequenced. The PBR gene is located in the q13.3 region of the long arm of human chromosome 22. The PBR play a major role in steroidogenesis, controlling cholesterol mitochondrial transport. Diazepam-binding inhibitor and its processing products, as well as porphyrins, have been suggested as putative endogenous ligands for these receptors. The PBR ligands have been shown to control cell proliferation and differentiation, and the binding capacity for these ligands is enhanced in some malignant tumors. Stress has been demonstrated to affect PBR bidirectionally. Acute stress is associated with increased PBR density, whereas chronic stress down-regulates PBR. **Key Words:** Peripheral-type benzodiazepine receptor—Central-type benzodiazepine receptor—Steroids—Cancer—Stress.

Benzodiazepines (BZs) are used clinically as muscle relaxants, anticonvulsants, anxiolytics, and sedative hypnotics. These effects are mediated via central-type BZ receptors (CBR) located in the central nervous system (CNS) (1,2). It has been found that binding of various BZs to CBR correlates with their clinical potency as

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anticonvulsants and anxiolytics (3). Pharmacological evidence suggests that BZs exert their therapeutic effects by facilitating synaptic actions of γ -aminobutyric acid (GABA) (4–6). Many attempts have been made to discover the endogenous ligand for CBR. It has been suggested by Guidotti et al. (7) that the endogenous ligand for CBR is a polypeptide of 105 amino acids: diazepam-binding inhibitor (DBI), which is both convulsant and anxiogenic.

Binding studies have shown that GABA and related agents enhance specific binding of the BZs [3 H]diazepam and [3 H]flunitrazepam to CBR in the membrane-bound state (8–12) as well as in the soluble state (13,14). The solubilization of these receptors was an important step in the characterization and partial purification (13–17) of the GABA/BZ receptor complex. This complex has been purified from bovine cerebral cortex, and the binding activity for BZs, barbiturates, β -carbolines, picrotoxin, and GABA was preserved after purification (15,16). Basically, the GABA/BZ receptor complex is composed of two α subunits with molecular mass of 53 kilodaltons (kDa), which contain the BZ binding site, and two β subunits with molecular mass of 57 kDa, which contain the binding site for GABA. Various parts of the inner side of the $\alpha_2\beta_2$ subunits structure form the chloride ion channel (15,16). Complementary DNA (cDNA) has been cloned and functional receptors expressed in frog oocyte (17).

In addition to CBR located in the CNS, specific recognition sites for BZs, called peripheral-type BZ receptors (PBR), have also been identified. The PBR are sparse in nervous tissue, but are prominent in various other organs (18–22). The PBR differ from CBR in their distribution within the brain, their lack of coupling to the GABA receptors, and their specificity to ligand binding. Clonazepam, which binds with high affinity to CBR, has very low affinity to PBR, whereas the reverse is true with regard to the BZ ligand Ro 5-4864 and the non-BZ ligand PK 11195, an isoquinoline carboxamide derivative (23). Diazepam and flunitrazepam exhibit moderate affinity to PBR (19–21).

PBR DISTRIBUTION

Anholt et al. (24) investigated the subcellular localization of PBR in rat adrenal gland. They found that the autoradiographic pattern of [3 H]PK 11195 binding sites in tissue sections of adrenal gland is similar to the distribution of cytochrome oxidase and monoamine oxidase (MAO), both of which are mitochondrial marker enzymes. The fact that the recovery of [3 H]PK 11195 binding in the nuclear, mitochondrial, microsomal, and soluble fractions does not correlate with markers for nuclei, lysosomes, peroxisomes, endoplasmic reticulum, plasma membrane, or cytoplasm, but does correlate with cytochrome oxidase activity, indicates that PBR are associated with mitochondria (24). It has also been found that PBR and MAO, but not cytochrome oxidase, are released simultaneously from isolated mitochondria by the detergent digitonin, which indicates that PBR are located on the mitochondrial outer membrane (24). Schoemaker et al. (25) reported that PBR binding in the rat brain is preferentially enriched in the nuclear fraction. Marangos et al. (26) found about fourfold enrichment in nuclear PBR specific binding compared to rat brain homogenate and twofold enrichment compared to the mito-

chondrial fraction. Basile and Skolnick (27) reported that the highest levels of [3 H]Ro 5-4864 binding were found in the crude nuclear, mitochondrial, and synaptosomal fractions. The last two reports, as well as others, showed that CBR density is highest in the synaptosomal fraction.

Mukherjee and Das (28) reported that PBR in the guinea pig lung are predominantly localized in the mitochondrial inner membrane. However, Obeirne et al. (29) demonstrated that PBR in rat liver are located on the outer mitochondrial fraction as well as on a nonmitochondrial fraction. Furthermore, Olson et al. (30) demonstrated PBR in red blood cells, which lack mitochondria; therefore, PBR might also be in a nonmitochondrial fraction.

Antkiewicz-Michaluk et al. (31) found that PBR in testis, lung, kidney, heart, skeletal muscle, liver, and brain subfractionate in a manner nearly identical to that of the mitochondrial enzyme succinate dehydrogenase. Most researchers in the field agree that PBR are localized mainly on the outer mitochondrial membrane.

SPECIES DIFFERENCES IN PBR

The binding of [3 H]Ro 5-4864 and [3 H]PK 11195 to the brain and various peripheral tissues has been studied (32-37). Awad and Gavish (32) found that [3 H]PK 11195 bound with high affinity to rat and calf cerebral cortical and kidney membranes and that [3 H]Ro 5-4864 also successfully labeled rat cerebral cortical and kidney membranes. In calf cerebral cortical and kidney membranes, however, the binding capacity of [3 H]Ro 5-4864 was only 3 and 4%, respectively, of that of [3 H]PK 11195. Displacement studies have shown that unlabeled Ro 5-4864, diazepam, and flunitrazepam are much more potent in displacing [3 H]PK 11195 from rat cerebral cortex and kidney membranes than from calf tissues. The rank order of potency of unlabeled Ro 5-4864 in displacing [3 H]PK 11195 from the cerebral cortex of other species was rat = guinea pig > cat = dog > rabbit > calf (32). After analysis of displacement curves, Ro 5-4864 was found to bind to two populations of binding sites from rat and calf kidney and from rat, guinea pig, rabbit, and calf cerebral cortex but to a single population of binding sites from cat and dog cerebral cortex. When [3 H]PK 11195 was used as a ligand, the rank order of binding capacity in cerebral cortex of these species was cat > calf > guinea pig > rabbit > dog > rat, whereas when [3 H]Ro 5-4864 was used, the rank order of binding capacity was cat > guinea pig > rat > rabbit > calf > dog (32).

Similar differences in the binding of [3 H]Ro 5-4864 and [3 H]PK 11195 have also been obtained in rat and calf pineal gland (33). The potency of Ro 5-4864 in inhibiting [3 H]PK 11195 binding to cat brain sections and membranes has been found to be 140 times lower than that of PK 11195 (34). Differences in PBR densities and distribution in the brain of various species have been shown using autoradiographic techniques (35). The binding of [3 H]Ro 5-4864 and [3 H]PK 11195 has also been determined in human tissues: [3 H]PK 11195 bound with nanomolar affinity to human cerebral cortex, kidney, and colon membranes; the specific binding of [3 H]Ro 5-4864, on the other hand, was barely detectable (36). Unlabeled PK 11195 was two orders of magnitude more potent than unlabeled Ro 5-4864 in displacing [3 H]PK 11195 specific binding from human cerebral cortex

and kidney membranes (36). These differences may have importance for the evolution of these receptors in various species.

Parola and Larid (37) reported that bovine PBR are pharmacologically and biochemically distinct from rat PBR, but that the isoquinoline site molecular weight is similar in both species. The species differences in Ro 5-4864 and PK 11195 binding are retained after solubilization (38), which indicates that the differences are due to variations in the molecular structure of PBR rather than to differences in membrane environment.

BIOCHEMICAL STUDIES ON PBR

PBR have been solubilized by Triton X-100 from rat kidney, but the detergent concentration had to be lowered by Bio-Beads (39). The PBR have also been solubilized by digitonin from rat kidney (40) and adrenal gland (41). Sodium cholate was found appropriate for PBR solubilization from rat kidney, but only after the addition of soybean lipids (42). 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) has been found suitable for PBR solubilization from cat brain in the presence of NaCl (43). Solubilization of active PBR enables purification of intact protein.

PK 14105 is an isoquinoline carboxamide that was synthesized by Doble et al. (44). This ligand labels PBR with high affinity, and after ultraviolet (UV) irradiation, it is attached covalently to the isoquinoline site (44). Using [^3H]PK 14105, various studies have found that the molecular mass of the isoquinoline site is about 18 kDa (44–46). [^3H]Flunitrazepam also labels PBR covalently after UV irradiation, but the molecular mass of the subunit labeled is 30 kDa (47).

[^3H]AHN 086 is an isothiocyanate derivative of the BZ Ro 5-4864 (48). This ligand can label a different subunit in PBR with a molecular mass of approximately 30 kDa (49). McEnery et al. (47) isolated rat mitochondrial BZ receptors that contained three subunits: an 18-kDa subunit, the site for isoquinoline; a 30-kDa subunit, labeled by [^3H]flunitrazepam, identified as adenine nucleotide carrier; and a 32-kDa subunit, labeled by [^3H]AHN 086, the voltage-dependent anion channel.

The site for isoquinoline has been purified to apparent homogeneity. Rat adrenal gland mitochondrial membranes have been covalently labeled with [^3H]PK 14105 and solubilized by digitonin. The soluble PBR complex covalently labeled by [^3H]PK 14105 has been purified by ion-exchange chromatography and reverse-phase high-performance liquid chromatography. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis shows a single band labeled by [^3H]PK 14105 with a molecular mass of 17 kDa (45). The cDNA coding for rat PBR has been isolated. The deduced amino acid sequence corresponds to a protein of 169 amino acids, which have five putative transmembrane regions (50). The amino acid sequence of the human PBR, deduced from corresponding cDNA, has been found to be 79% identical to that reported for rat PBR. Using the cDNA of human PBR as a probe, the gene for PBR has been located in the q13.3 region of the long arm of human chromosome 22 (51).

Cloned bovine cDNA is 821 nucleotide bases in length and encodes for a protein

of 169 amino acids (52). Comparison of the deduced amino acid sequences has revealed that the isoquinoline site of bovine cDNA is 78% identical to that of rat (52). There are three regions in which the variability is higher. Both rat and bovine isoquinoline sites have similar predicted secondary structures, with five putative membrane-spanning regions as determined from hydropathy plots of the primary sequences (52).

PBR AND MALIGNANCY

Benzodiazepines control proliferative and differentiating processes of normal and malignant cells, as shown by several experimental models. The BZs enhance melanogenesis in B16/C3 melanoma cells (53) and inhibit the proliferation of thymoma cells in vitro at micromolar concentrations (54). They also inhibit nerve-growth-factor-induced neurite outgrowth in PC12 cell cultures (55). Specific stimulation of *C-fos* protooncogenes by nerve growth factor has been found in the presence of BZs (56). At nanomolar concentrations, PBR ligands stimulate [³H]thymidine incorporation into DNA and increase cell proliferation in C6 glioma (57). In most of these observations, there is a correlation between BZ affinity and potency to regulate their effect, which indicates that PBR are involved in the control of cell growth and differentiation in vitro. The PBR binding increases significantly in experimental animal brain tumors (58,59) and in brain tumors in humans (60). In human brains at postmortem examination, [³H]PK 11195 binds mainly to glioma or astrocytoma (58).

The PBR density and affinity in ovarian carcinoma (61) and colonic adenocarcinoma (62) have been compared to those in normal tissues. There was no difference in the affinity for PK 11195, but there was a two- to threefold increase in the density of PBR in ovarian carcinoma and in colonic adenocarcinoma as compared to that of normal control tissues (61,62). This increase in PBR density may be related to higher metabolic rates in neoplasms (63) or, alternatively, may reflect alteration in the structure of mitochondria in the tumor, termed "mitochondrial pyknosis," which is revealed by microscopic ultrastructural examination (64).

THE FUNCTION OF PBR IN STEROIDOGENESIS

Localization in Steroidogenic Organs

Endocrine organs are especially enriched in PBR. In the adrenal, the receptor is localized in the cortex and absent in the medulla (65,66). In the testis, PBR are present mainly in the interstitial tissue (65). The presence of the receptor has also been detected in human term placenta, a tissue also active in steroidogenesis (67). The PBR in adrenal cortex and testis are dependent on the secretion of pituitary trophic hormones. Hypophysectomy causes depletion of PBR in adrenal and testis, and the reduction in PBR correlates with the atrophy of these organs (68). Adrenocorticotrophic hormone (ACTH) administration to hypophysectomized rats induces an increase in PBR density (69). It has been suggested that PBR play a role in steroidogenesis, because ligands active at these receptors stimulate the

production of steroids in human term placental explants (70) and adrenocortical tissue (71).

PBR and Mitochondrial Cholesterol Transport

The PBR regulate the steroid hormone production induced by pituitary trophic hormones. Pituitary peptide hormones such as gonadotropins and ACTH activate adenylate cyclase and the production of adenosine 3',5'-cyclic monophosphate in the target cells. Subsequently, cholesterol is converted into pregnenolone by cytochrome P-450 side-chain-cleavage (P-450_{scc}) enzyme, which is located on the inner mitochondrial membrane (for review see ref. 72). The transport of cholesterol from the outer to the inner mitochondrial membrane is rate limiting in steroid hormone biosynthesis (73). Luteinizing hormone (LH), ACTH, and follicle-stimulating hormone activate cholesterol transport via the mitochondrial membrane (74,75). The BZ 4'-chlorodiazepam (Ro 5-4864) enhances cholesterol transport from cell cytoplasm to inner mitochondrial membrane (76).

It appears that PBR mediate the translocation of cholesterol to the inner mitochondrial membrane (73). The potency of PBR ligands to stimulate steroidogenesis in Y-1 adrenocortical cells correlates with their potency to inhibit [³H]PK 11195 binding to PBR (77). These observations prove that PBR ligands control steroid production when they bind to mitochondrial BZ recognition sites. The stimulation of steroid biosynthesis by PBR ligands has also been demonstrated in Leydig cells (78) and granulosa cells (79). In these steroidogenic tissues, a high correlation was demonstrated between steroidogenic potency and the affinity of PBR ligands to their receptor. It was shown that PBR regulate cholesterol uptake from the cytoplasmic stores into mitochondria, promoting cholesterol availability to the P-450_{scc} enzyme (78). The polypeptide DBI, an endogenous ligand for PBR that is present in steroidogenic tissues, can activate steroidogenesis via PBR stimulation (74). Flunitrazepam inhibits the steroidogenesis induced by trophic hormones in adrenocortical and Leydig cell lines (75). The antagonistic activity of steroidogenesis is mediated through its interaction with the PBR located in the mitochondria.

Putative Endogenous Ligand for PBR

DBI and Its Processing Products

The intensive research elucidating the role of PBR in the regulation of mitochondrial steroidogenesis stimulated the search for endogenous ligands that mediate the actions of ACTH and LH. Several naturally occurring substances that are active at the PBR have been identified, including the peptide DBI and its processing products (80). The DBI is a polypeptide with a molecular mass of 9 kDa, which was isolated first from rat brain and was shown to displace [³H]diazepam from both CBR and PBR (80-82). In rat and human brain, the highest DBI-like immunoreactivity and DBI messenger RNA (mRNA) were identified in the cerebellum and hypothalamus, whereas the lowest concentrations of DBI were shown in the striatum (80-82). In the peripheral organs, the liver was shown to be

enriched with DBI, and smaller amounts were detected in the duodenum, testis, kidney, adrenal cortex, skeletal muscle, and spleen. The cells of the zona glomerulosa in the adrenal are enriched with DBI, and in the testis, extremely high concentrations of DBI are detectable in Leydig cells. In the brain, DBI is especially abundant in astroglial cells (80).

Two naturally occurring DBI-processing products have been identified in the rat brain: octadecaneuropeptide (ODN) and triakontateraneuropeptide (TTN). Although the structures of the two peptides overlap, they differ in their chemical properties and biological activity profiles. ODN binds preferentially to CBR, TTN binds preferentially to PBR (80). The amount of DBI and its rate of synthesis in the adrenal cortex are under the control of ACTH (83); DBI and its active products regulate steroidogenesis activated by ACTH and LH via binding to PBR (74). The major human DBI gene has been assigned to the q12-21 region of chromosome 2 (80).

Porphyrins

Verma et al. (84) demonstrated that naturally occurring porphyrins (protoporphyrin IX, mesoporphyrin X, deuteroporphyrin IX, and hemin) possess high affinity ($K_i = 14\text{--}40$ nM) for PBR. The high affinity of these porphyrins for PBR suggests their role as putative endogenous ligands for these receptors. The precursors as well as the breakdown products of porphyrins exhibit low affinity to PBR (85).

PBR in Various Peripheral Organs

Endocrine Organs

Male genital tract. High density of PBR has been visualized by autoradiography in the interstitial tissue of the testis (65). The PBR have also been identified in vas deferens, prostate, seminal vesicle, and Cowper's gland (86), and PBR ligands increase both basal and human chorionic gonadotropin-stimulated testosterone secretion from decapsulated testis (87,88). Testicular PBR are dependent on the trophic influence of pituitary hormones (68) and on the gonadal hormone testosterone (89). Administration of the antiandrogenic agent cyproterone acetate depletes testicular PBR (89). Orchiectomy induces reduction of PBR density in Cowper's gland, and testosterone replacement prevents castration-induced PBR depletion (90). Administration of the female gonadal hormone estradiol-17 β , similar to cyproterone acetate, also depletes the expression of PBR in the testis (91). Thus, it seems that PBR in the testis and accessory sex organs are hormone dependent.

Female genital tract. The PBR have been localized in the rat ovary, in granulosa cells, and in interstitial theca cells, as well as in the uterus, the oviduct, and the mammary gland (72,92). In the immature rat ovary, PBR density increases with age (92). Removal of pituitary prevents this age-dependent upregulation of PBR and causes depletion of ovarian PBR (93). The effect of hypophysectomy on ovarian PBR can be prevented by gonadotropin or estrogen treatment (92). The expression of ovarian PBR is altered in the adult female rat by the menstrual cycle

and reaches a maximal level on the day of proestrus, when follicular size is maximal and the follicle is ready to ovulate (94). It seems that PBR density in the ovary increases with granulosa differentiation and is associated with follicular growth and development. In human ovarian granulosa cells, PBR density is higher in mature cells (95). Thus, it appears that ovarian steroidogenic activity is regulated by PBR (79), and the expression of PBR is controlled by pituitary and gonadal sex hormones.

In the oviduct and uterus, PBR are also dependent on female sex hormones (92-94). In human uterine endometrium, the density of PBR has been demonstrated to be dependent on the level of estradiol-17 β (72), which indicates that uterine PBR are under female hormonal regulation. The PBR-specific ligands also affect oxytocin-induced contractions in rat uterine tissue (72).

Thyroid and Pineal Gland

In the heart, kidney, and testis, PBR are sensitive to thyroxine. Chronic administration of thyroxine to rats induces upregulation of cardiac, renal, and testicular PBR (96). The response of PBR to thyroxine may reflect a compensatory adaptive mechanism to experimental hyperthyroidism. Thyroid hormones as well as PBR ligands are involved in steroidogenesis (97). The metabolism of thyroid hormones and the expression of PBR are regulated by endogenous steroids (96,98). The possibility of a bidirectional effect of thyroid hormones on PBR (e.g., upregulation by hyperthyroidism and downregulation by hypothyroidism) merits further investigation.

The PBR have been identified in rat and human pineal gland and are suggested to be associated with catecholamine nerve terminal (99,100). Benzodiazepines amplify norepinephrine-induced melatonin synthesis via the PBR (101). Because melatonin plays a role in anxiety and affective disorders, it is possible that pineal PBR are involved in neuroendocrine and emotional responses to stress. This possibility is supported by the observation that both deprivation of sympathetic innervation (superior cervical ganglionectomy) and a 3-week exposure to constant light selectivity downregulate pineal PBR (100).

Adrenal Gland

The highest densities of PBR are found in the adrenal cortex (65). The adrenal PBR are dependent on the trophic effect of ACTH, and hypophysectomy induces a dramatic depletion of PBR in the zona fasciculata and zona reticularis (68,69). Cyproterone acetate treatment induces depletion of adrenal PBR, possibly due to the suppression of pituitary ACTH secretion caused by this antiandrogenic drug (89). The PBR play a significant role in the function of the hypothalamic-pituitary-adrenal axis. The PBR-specific ligand Ro 5-4864 enhances the secretion of corticotropin-releasing hormone, whereas PK 11195 directly stimulates the release of ACTH from the pituitary (102). This line of evidence indicates that PBR-specific ligands stimulate adrenal cortical steroidogenic activity by activating the hypothalamic-pituitary-adrenal axis. The PBR-specific ligands stimulate adrenal cell steroidogenic activity by increasing cholesterol transport from the intracellular stores to the mitochondrial P-450_{sc} enzyme (77). Endogenous PBR

ligands (DBI and its active compounds) stimulate steroidogenesis (74). Thus, it seems that these receptors play a role in the neuroendocrine response to stress (103).

Kidney

The PBR in the kidney are especially rich in the ascending limb of the loop of Henle, the distal convoluted tubule, and the collecting tubules (104). In these regions, sodium and chloride transport as well as water reabsorption occur. The distribution of PBR in the kidney is similar to that of angiotensin II and aldosterone. Adrenalectomy induces reduction in renal PBR, which is reversed by administration of aldosterone (105). Acute exposure to angiotensin II causes an immediate dose-dependent decrease in rat renal PBR (106). The downregulation induced by angiotensin II is similar to that obtained following prolonged inescapable shock stress (107). Thus, it seems that the renin-angiotensin axis plays a pivotal role in the rapid stress-induced decrease in renal PBR.

Progesterone administration has also been reported to alter renal PBR (108). Deoxycorticosterone and hydrocortisone treatments cause an increase in PBR density in this organ (69,109). These results support the assumption that the renal PBR are dependent on an intact hypothalamic-pituitary-adrenal axis.

The effect of various procedures on PBR is summarized in Table 1.

The Involvement of PBR in Anxiety and Stress

Acute Stress

The involvement of PBR in acute stress is well documented in the literature. Drugan et al. (107) were the first to demonstrate an increase in renal PBR following exposure to five tail shocks. Later, a similar finding was reported by Basile et

TABLE 1. *Effects of various procedures on the expression of PBR in different organs*

Organ/procedure	PBR	Reference (No.)
Testis		
Hypophysectomy	↓	Anholt et al., 1985 (68)
Estradiol	↓	Gavish et al., 1986 (91)
Cyproterone	↓	Amiri et al., 1991 (89)
Cowper's gland		
Orchiectomy	↓	Weizman et al., 1992 (90)
Ovary		
Hypophysectomy	↓	Bar-Ami et al., 1989 (93)
Pineal gland		
Ganglionectomy	↓	Weissman et al., 1984 (100)
Constant light	↓	Weissman et al., 1984 (100)
Adrenal gland		
Hypophysectomy	↓	Anholt et al., 1985 (68)
		Fares et al., 1989 (69)
Cyproterone acetate	↓	Amiri et al., 1991 (89)
Kidney		
Deoxycorticosterone	↑	Regan et al., 1981 (109)
Adrenalectomy	↓	Basile et al., 1985 (105)
Hydrocortisone	↑	Fares et al., 1989 (69)
Angiotensin	↓	Holmes and Drugan, 1992 (106)

al. (110), who showed an increase in PBR in mouse cerebral cortex and cardiac ventricles after acute maximal electroshock. Laparotomy in rats induced increased PBR binding in the cerebral cortex 1 and 3 days after the procedure, whereas 7 days after the surgery, PBR values returned to normal levels; similar effects were obtained in the kidney (111).

Forced swimming stress, which is another model of acute stress, has also been reported to elevate PBR density in rats (112–114). Rágo et al. (114) reported that the pretreatment of rats with β -(phenyl) GABA, a GABA agonist, almost completely prevented the stress-induced increase in platelet PBR. This protective effect may be related to the mild sedative and tranquilizing activity of the GABA agonist.

Recently, two additional studies on the effect of acute stress on PBR demonstrated an increase following noise burst (103) and conditioned fear (115). The relationship of PBR and acute stress in humans was studied by Karp et al. (116), who demonstrated elevated platelet receptor density in medical residents immediately after specialization examinations.

The effect of acute stress on the expression of PBR in various organs is summarized in Table 2.

TABLE 2. *Effect of acute stress on PBR in different organs*

Type of stress/organ	PBR	Reference (No.)
Inescapable shocks (rats)		
Kidney	↑	Drugan et al., 1986 (107)
Cerebral cortex	—	
Acute maximal electroshock (mice)		
Cerebral Cortex	↑	Basile et al., 1987 (110)
Heart	↑	
Cerebellum	—	
Hippocampus	—	
Kidney	—	
Laparotomy (rats)		
Cortex		
Day 1	↑	Okun et al., 1988 (111)
Day 3	↑	
Day 7	—	
Kidney		
Day 1	↑	
Day 3	↑	
Day 7	—	
Examination (humans)		
Platelets	↑	Karp et al., 1989 (116)
Swimming stress (rats)		
Olfactory bulb	↑	Novas et al., 1987 (112)
Kidney	↑	
Swimming stress (rats)		
Kidney	↑	Rágo et al., 1989 (113)
Swimming stress (rats)		
Platelets	↑	Rágo et al., 1990 (114)
Noise burst (rats)		
Adrenal gland	↑	Ferrarese et al., 1991 (103)
Conditioned fear (rats)		
Olfactory bulb	↑	Holmes et al., 1992 (115)

Chronic Stress

Drugan et al. (107) examined the effect of exposure to 80 tail shocks on the expression of PBR in rats. The repeated stress induced a significant decrease of PBR density in cerebral cortex, pituitary gland, heart, and kidney, but did not affect the density of hippocampal, adrenal, and pulmonary PBR. Hypophysectomy, adrenalectomy, and chemical sympathectomy (using 6-hydroxydopamine) did not influence the stress-induced decrease in renal PBR (117). Thus, it seems that adrenal hormones as well as peripheral catecholamines do not mediate the modulatory effect of chronic stress on PBR expression.

In order to evaluate involvement of the CNS in the stress-induced downregulation of PBR, clonazepam and sodium pentobarbital were administered to rats prior to repeated tail shocks. Clonazepam attenuated the PBR response to stress, whereas pentobarbital completely blocked the stress-induced downregulation of renal PBR. Because both agents are active at the CBR, it seems that both CBR and PBR play a role in the response to stress (118,119). It has been suggested that endogenous BZ ligands active at both CBR and PBR, which are released during stress (103), modulate the GABA-gated chloride conductance and can also activate the biosynthesis and local release of GABA-active steroids by oligodendrocytes (120).

The interaction between chronic stress and PBR has also been investigated in Maudsley reactive (MR) rats. These rats have been selectively bred for a high level of fearfulness, in contrast to Maudsley nonreactive rats, which exhibit low levels of fearfulness. The MR rats displayed reduced PBR density in the kidney and heart, but not in other examined organs (121).

The effect of metabolic stress has been studied in rats deprived of food for 5 days (122). Reduced PBR density was observed in the adrenal gland, kidney, and heart, whereas the hypothalamus and ovary were unaffected. The decrease in renal and cardiac PBR can be attributed to the stress-induced downregulation of PBR, which is in accordance with a previous report (107), or to metabolic and endocrine changes occurring during starvation. Following a 5-day refeeding period, renal, and cardiac PBR returned to normal values, whereas adrenal PBR remained reduced.

In order to evaluate the effect of long-term repeated stress in humans, platelet PBR were measured in healthy soldiers undergoing a parachute-training course (123). Similar to the stress-induced downregulatory effect of PBR reported in laboratory models (107), in humans, a significant decrease in platelet PBR was observed after the fourth daily parachute jump. Table 3 summarizes the effect of chronic stress on the expression of PBR.

At this point, it seems that one can conclude that PBR are sensitive to stress. Moreover, acute stress has been demonstrated to upregulate PBR in various organs, and chronic stress downregulates this receptor.

CONCLUSION

The PBR have a pivotal role in steroidogenesis and regulate cell differentiation and proliferation. These receptors are sensitive to hormonal changes and stress.

TABLE 3. *Effect of chronic stress on PBR in different organs*

Type of stress/organ	PBR	Reference (No.)
Eighty tail shocks (rats)		
Cerebral cortex	↓	
Pituitary	↓	
Heart	↓	
Kidney	↓	
Hippocampus	—	
Adrenal gland	—	
Lung	—	Drugan et al., 1986 (107)
Maudsley reactive (rats)		
Kidney	↓	
Heart	↓	
Cerebral cortex	—	
Hippocampus	—	
Hypothalamus	—	
Lung	—	
Pituitary	—	
Adrenal gland	—	Drugan et al., 1987 (121)
Food deprivation (rats)		
Adrenal	↓	
Kidney	↓	
Heart	↓	
Hypothalamus	—	
Ovary	—	Weizman et al., 1990 (122)
Parachuting (humans)		
Platelets	↓	Dar et al., 1991 (123)

The increased PBR density following exposure to acute stress may reflect activation of the hypothalamic-pituitary-adrenal axis. In contrast, the decrease in PBR observed following repeated stress may reflect an adaptive mechanism that prevents long-term hypercortisolemia.

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REFERENCES

1. Braestrup C, Squires RF. Specific benzodiazepine receptors in rat brain characterized by high-affinity [³H]diazepam binding. *Proc Natl Acad Sci USA* 1977;74:3805-9.
2. Mohler H, Okada T. Benzodiazepine receptors: demonstration in the central nervous system. *Science* 1977;198:849-51.
3. Mohler H, Okada T, Heitz PH, Ulrich J. Biochemical identification of the site of action of benzodiazepines in human brain. *Life Sci* 1978;22:985-96.
4. Haefely W, Kulcsar A, Mohler H, Pieri L, Polc P, Schaffner R. Possible involvement of GABA in the central action of benzodiazepines. In: Costa E, Greengard P, eds. *Mechanisms of action of benzodiazepines*. New York: Raven Press, 1975:131-51.
5. MacDonald R, Barker JL. Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurons. *Nature* 1978;271:563-4.
6. Costa E, Guidotti A. Molecular mechanisms in the receptor action of benzodiazepines. *Annu Rev Pharmacol Toxicol* 1979;19:531-45.
7. Guidotti A, Forchetti CM, Corda MG, Kondel D, Bennett CD, Costa E. Isolation, characterization, and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. *Proc Natl Acad Sci USA* 1983;80:3531-5.
8. Briley M, Langer SZ. Influence of GABA receptor agonists and antagonists on the binding of [³H]diazepam to the benzodiazepine receptor. *Eur J Pharmacol* 1978;52:129-32.

9. Martin IL, Candy JM. Facilitation of benzodiazepine binding by sodium chloride and GABA. *J Neuropharmacol* 1978;17:993-8.
10. Tallman JF, Thomas JW, Gallager DW. GABAergic modulation of benzodiazepine binding site sensitivity. *Nature* 1978;274:383-5.
11. Wastek GJ, Speth RC, Reisine TD, Yamamura HI. The effect of γ -aminobutyric acid on [3 H]flunitrazepam binding in rat brain. *Eur J Pharmacol* 1978;50:445-7.
12. Karobath M, Sperk G. Stimulation of benzodiazepine receptor binding by γ -aminobutyric acid. *Proc Natl Acad Sci USA* 1979;76:1004-6.
13. Gavish M, Snyder SH. Soluble benzodiazepine receptors: GABAergic regulation. *Life Sci* 1980;26:579-82.
14. Sherman-Gold R, Dudai Y. Solubilization and properties of a benzodiazepine receptor from calf cortex. *Brain Res* 1980;198:485-90.
15. Casalotti SO, Stephenson FA, Barnard EA. Separate subunits for agonist and benzodiazepine binding in the γ -aminobutyric acid_A receptor oligomer. *J Biol Chem* 1986;261:15013-6.
16. Mamalaki C, Stephenson FA, Barnard EA. The GABA_A/benzodiazepine receptor is a heterotetramer of homologous α and β subunits. *EMBO J* 1987;6:561-5.
17. Schofield PR, Darlison MG, Fugita N, et al. Sequence and functional expression of the GABA_A receptor shows a ligand-gated receptor super-family. *Nature* 1987;328:221-7.
18. Gavish M, Katz Y, Bar-Ami S, Weizman R. Biochemical, physiological and pathological aspects of the peripheral benzodiazepine receptor. *J Neurochem* 1992;58:1589-601.
19. Davies LP, Huston V. Peripheral benzodiazepine binding sites in heart and their interaction with dipyridamole. *Eur J Pharmacol* 1981;73:209-11.
20. Taniguchi T, Wang JKT, Spector S. [3 H]Diazepam binding sites on rat heart and kidney. *Biochem Pharmacol* 1982;31:589-90.
21. Wang JKT, Taniguchi T, Spector S. Properties of [3 H]diazepam binding on rat blood platelets. *Life Sci* 1980;27:1881-8.
22. Schoemaker H, Bliss M, Yamamura HI. Specific high-affinity saturable binding of [3 H]Ro 5-4864 to benzodiazepine binding sites in the rat cerebral cortex. *Eur J Pharmacol* 1981;71:173-5.
23. Le Fur, Perrier ML, Vaucher N. Peripheral benzodiazepine binding sites: effect of PK 11195, 1-(2-chlorophenyl)-N-(1-methylpropyl)-3-isoquinoline carboxamide. *Life Sci* 1983;32:1839-47.
24. Anholt RRH, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor: localization to the mitochondrial outer membrane. *J Biol Chem* 1986;261:576-83.
25. Schoemaker H, Richard G, Boles W, Dale H, Yamamura HI. Specific high-affinity binding sites for [3 H]Ro 5-4864 in rat brain and kidney. *J Pharmacol Exp Ther* 1983;225:61-9.
26. Marangos PJ, Patel J, Boulenger JP, Clark-Rosenberg R. Characterization of peripheral-type benzodiazepine binding sites in brain using [3 H]Ro 5-4864. *Mol Pharmacol* 1982;22:26-32.
27. Basile AS, Skolnick P. Subcellular localization of "peripheral-type" binding sites for benzodiazepines in rat brain. *J Neurochem* 1986;46:305-8.
28. Mukherjee S, Das SK. Subcellular distribution of "peripheral-type" binding sites for [3 H]Ro 5-4864 in guinea pig lung: localization to the mitochondrial inner membrane. *J Biol Chem* 1989;264:713-6, 718.
29. O'Beirne GB, Woods MJ, Williams DC. Two subcellular locations for peripheral-type benzodiazepine acceptors in rat liver. *Eur J Biochem* 1990;188:131-8.
30. Olson JMM, Ciliax BJ, Mancini WR, Young AB. Presence of peripheral-type benzodiazepine binding sites on human erythrocyte membranes. *Eur J Pharmacol* 1988;152:47-53.
31. Antkiewicz-Michaluk L, Guidotti A, Krueger KE. Molecular characterization and mitochondrial density of a recognition site for peripheral-type benzodiazepine ligands. *Mol Pharmacol* 1988;34:272-8.
32. Awad M, Gavish M. Binding of [3 H]Ro 5-4864 and [3 H]PK 11195 to cerebral cortex and peripheral tissues of various species: species differences and heterogeneity in peripheral benzodiazepine binding sites. *J Neurochem* 1987;49:1407-14.
33. Basile AS, Klein DC, Skolnick P. Characterization of benzodiazepine receptors in the bovine pineal gland: evidence for the presence of an atypical binding site. *Mol Brain Res* 1986;1:127-35.
34. Benavides J, Savaki HE, Malgouris C, et al. Autoradiographic localization of peripheral benzodiazepine binding sites in the cat brain with [3 H]PK 11195. *Brain Res Bull* 1984;13:69-77.
35. Cymerman V, Pazo SA, Palacios JM. Evidence for species differences in "peripheral" benzodiazepine receptors: an autoradiographic study. *Neurosci Lett* 1986;66:153-8.
36. Awad M, Gavish M. Peripheral-type benzodiazepine receptors in human cerebral cortex, kidney, and colon. *Life Sci* 1991;49:1155-61.
37. Parola AL, Larid HE II. The bovine peripheral-type benzodiazepine receptor: a receptor with low affinity for benzodiazepines. *Life Sci* 1990;48:757-64.

38. Awad M, Gavish M. Species differences and heterogeneity of solubilized peripheral-type benzodiazepine binding sites. *Biochem Pharmacol* 1989;38:3843-9.
39. Martini C, Giannaccini G, Lucacchini A. Solubilization of rat kidney benzodiazepine binding sites. *Biochim Biophys Acta* 1983;728:289-92.
40. Gavish M, Fares F. Solubilization of peripheral benzodiazepine binding sites from rat kidney. *J Neurosci* 1985;5:2889-93.
41. Benavides J, Menager J, Burgevin MC. Characterization of solubilized "peripheral-type" benzodiazepine binding sites from rat adrenal gland using [³H]PK 11195, an isoquinoline carboxamide derivative. *Biochem Pharmacol* 1985;34:167-70.
42. Anholt RRH, Aebi U, Pederson PL, Snyder SH. Solubilization and reassembly of the mitochondrial benzodiazepine receptor. *Biochemistry* 1986;25:2120-5.
43. Awad M, Gavish M. Solubilization of peripheral-type benzodiazepine binding sites from cat cerebral cortex. *J Neurochem* 1989;52:1880-5.
44. Doble A, Ferris O, Burgevin MC. Photoaffinity labeling of peripheral-type benzodiazepine binding sites. *Mol Pharmacol* 1987;31:42-9.
45. Antkiewicz-Michaluk L, Mukhin AG, Guidotti A, Krueger KE. Purification and characterization of a protein associated with peripheral-type benzodiazepine binding sites. *J Biol Chem* 1988;263:17317-21.
46. Skowronski R, Fanestil DD, Beaumont K. Photoaffinity labeling of peripheral-type benzodiazepine receptors in rat kidney mitochondria with [³H]PK 14105. *Eur J Pharmacol* 1988;148:187-93.
47. McEnery MW, Snowman AM, Trifiletti RR, Snyder SH. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci USA* 1992;89:3170-4.
48. Luddens HWM, Newman AH, Rice KC, Skolnick P. AHN 086: an irreversible ligand of "peripheral" benzodiazepine receptors. *Mol Pharmacol* 1986;29:540-5.
49. McCabe RT, Schoenheimer JA, Skolnick P, et al. [³H]AHN 086 acylates peripheral benzodiazepine receptors in the rat pineal gland. *FEBS Lett* 1989;244:263-7.
50. Sprengel R, Werner P, Seebury PH, et al. Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *J Biol Chem* 1989;264:20415-21.
51. Riond J, Mattei MGH, Kaghad M, et al. Molecular cloning and chromosomal localization of a human peripheral-type benzodiazepine receptor. *Eur J Biochem* 1991;195:305-11.
52. Parola AL, Stump DG, Pepper DJ, Krueger KE, Regan JW, Larid HE II. Cloning and expression of a pharmacologically unique bovine peripheral-type benzodiazepine receptor isoquinoline binding protein. *J Biol Chem* 1991;266:14082-7.
53. Matthew E, Laskin JD, Zimmerman EA, Weinstein IB, Hsu KC, Engelhardt DL. Benzodiazepines have high-affinity binding sites and induce melanogenesis in B16/C3 melanoma cells. *Proc Natl Acad Sci USA* 1981;78:3935-9.
54. Wang JKT, Morgan JI, Spector S. Benzodiazepines that bind at peripheral sites inhibit cell proliferation. *Proc Natl Acad Sci USA* 1984;81:753-6.
55. Morgan JI, Johnson MD, Wang JKT, Sonnenfeld KH, Spector S. Peripheral-type benzodiazepines influence ornithine decarboxylase levels and neurite outgrowth in PC12 cells. *Proc Natl Acad Sci USA* 1985;82:5223-6.
56. Currant T, Morgan JI. Superinduction of C-fos by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* 1985;229:1265-8.
57. Ikezaki K, Black KL. Stimulation of cell growth and DNA synthesis by peripheral benzodiazepine. *Cancer Lett* 1990;49:115-20.
58. Starosta-Rubinstein SR, Ciliax BJ, Penney JB, McKeever PE, Young AB. Imaging of a glioma using peripheral benzodiazepine receptor ligands. *Proc Natl Acad Sci USA* 1987;84:891-5.
59. Black KL, Ikezaki K, Arthur WT. Imaging of brain tumors using peripheral benzodiazepine ligands. *J Neurosurg* 1989;71:113-8.
60. Black KL, Ikezaki K, Santori EM, Becker DP, Vintens HV. Specific high-affinity binding to peripheral benzodiazepine receptor ligands to brain tumors in rat and human. *Cancer* 1990;68:93-7.
61. Katz Y, Ben-Baruch G, Kloog Y, Menczer J, Gavish M. Increased density of peripheral benzodiazepine-binding sites in ovarian carcinomas as compared with benign ovarian tumors and normal ovaries. *Clin Sci* 1990;78:155-8.
62. Katz Y, Eitan A, Gavish M. Increase in peripheral benzodiazepine binding sites in colonic adenocarcinoma. *Oncology* 1990;47:139-42.
63. Gold J. Cancer cachexia and gluconeogenesis. *Ann NY Acad Sci* 1974;230:103-11.
64. Ghadially FN. *Ultrastructural pathology of cell*. London: Butterworth, 1975:122-3.

65. De Souza EB, Anholt RRH, Murphy KMM, Snyder SH, Kuhar MJ. Peripheral-type benzodiazepine receptor in endocrine organs: autoradiographic localization in rat pituitary, adrenal, and testis. *Endocrinology* 1985;116:567-73.
66. Benavides J, Malgouris C, Imbault F, et al. Peripheral-type benzodiazepine binding sites in rat adrenal: binding studies with [³H]PK 11195 and autoradiographic localization. *Arch Int Pharmacodyn Ther* 1983;226:38-49.
67. Fares F, Gavish M. Characterization of peripheral benzodiazepine binding sites in human term placenta. *Biochem Pharmacol* 1986;35:227-30.
68. Anholt RRH, De Souza EB, Kuhar MJ, Snyder SH. Depletion of peripheral-type benzodiazepine receptors after hypophysectomy in rat adrenal gland and testis. *Eur J Pharmacol* 1985;110:41-6.
69. Fares F, Bar-Ami S, Haj-Yehia Y, Gavish M. Hormonal regulation of peripheral benzodiazepine binding sites in female rat adrenal gland and kidney. *J Receptor Res* 1989;9:143-57.
70. Barnea ER, Fares F, Gavish M. Modulatory action of benzodiazepines on human term placental steroidogenesis in vitro. *Mol Cell Endocrinol* 1989;64:155-9.
71. Yanagibashi K, Ohno Y, Nakamichi N, et al. Peripheral-type benzodiazepine receptors are involved in the regulation of cholesterol side chain cleavage in adrenocortical mitochondria. *J Biochem (Tokyo)* 1989;106:1026-9.
72. Gavish M, Bar-Ami S, Weizman R. The endocrine system and mitochondrial benzodiazepine receptors. *Mol Cell Endocrinol* 1992;88:1-13.
73. Krueger KE, Papadopoulos V. Peripheral type benzodiazepine receptors modulate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. *J Biol Chem* 1990;265:15015-21.
74. Papadopoulos V, Berkovich A, Krueger KE. The role of diazepam binding inhibitor and its processing products at mitochondrial benzodiazepine receptors: regulation of steroid biosynthesis. *Neuropharmacology* 1991;30:1417-23.
75. Papadopoulos V, Nowzari FB, Krueger KE. Hormone-stimulated steroidogenesis is coupled to mitochondrial benzodiazepine receptors. *J Biol Chem* 1991;266:3682-7.
76. Besman MJ, Yanagibashi K, Lee TD, Kawamura M, Hall PE, Shively JE. Identification of des-(Gly-Ile)-endozepine as an effector of corticotropin-dependent adrenal steroidogenesis: stimulation of cholesterol delivery is mediated by the peripheral benzodiazepine receptor. *Proc Natl Acad Sci USA* 1989;86:4897-901.
77. Mukhin AG, Papadopoulos V, Costa E, Krueger KE. Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proc Natl Acad Sci USA* 1989;86:9813-16.
78. Papadopoulos V, Mukhin AG, Costa E, Krueger KE. The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis. *J Biol Chem* 1990;265:3772-9.
79. Amsterdam A, Suh BS. An inducible functional peripheral benzodiazepine receptor in mitochondria of steroidogenic granulosa cells. *Endocrinology* 1991;129:503-10.
80. Costa E, Guidotti A. Diazepam binding inhibitor (DBI): a peptide with multiple biological actions. *Life Sci* 1991;49:325-44.
81. Costa E, Corda MG, Guidotti A. On a brain polypeptide functioning as a putative effector for the recognition sites of benzodiazepine and beta-carboline derivatives. *Neuropharmacology* 1983;22:1481-92.
82. Guidotti A, Forchetti CM, Corda MG, Konkel D, Bennet CD, Costa E. Isolation, characterization, and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. *Proc Natl Acad Sci USA* 1983;80:3531-3.
83. Massotti M, Slobodyansky E, Konkel D, Costa E, Guidotti A. Regulation of diazepam binding inhibitor in rat adrenal gland by adrenocorticotropin. *Endocrinology* 1991;129:591-6.
84. Verma A, Nye JS, Snyder SH. Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptors. *Proc Natl Acad Sci USA* 1987;84:2256-60.
85. Snyder SH, Verma A, Trifiletti RR. The peripheral-type benzodiazepine receptor: a protein of mitochondrial outer membranes utilizing porphyrins as endogenous ligands. *FASEB J* 1987;1:282-8.
86. Katz Y, Amiri Z, Weizman A, Gavish M. Identification and distribution of peripheral benzodiazepine binding sites in male rat genital tract. *Biochem Pharmacol* 1990;40:817-20.
87. Ritta MN, Campos MB, Calandra RS. Effect of GABA and benzodiazepines on testicular androgen production. *Life Sci* 1987;40:791-8.
88. Ritta MN, Calandra RS. Testicular interstitial cells are target for peripheral benzodiazepines. *Neuroendocrinology* 1989;49:262-6.
89. Amiri Z, Weizman R, Katz Y, Lochner A, Gavish M. Testosterone and cyproterone acetate modulate peripheral but not central benzodiazepine receptors in rat. *Brain Res* 1991;553:155-8.
90. Weizman A, Amiri Z, Katz Y, Snyder SH, Gavish M. Testosterone prevents castration-induced

- reduction in peripheral benzodiazepine receptors in Cowper's gland and adrenal. *Brain Res* 1992;572:72-5.
91. Gavish M, Okun F, Weizman A, Youdim MBH. Modulation of peripheral benzodiazepine binding sites following chronic estradiol treatment. *Eur J Pharmacol* 1986;127:147-51.
 92. Fares F, Bar-Ami S, Brandes JM, Gavish M. Gonadotropin- and estrogen-induced peripheral-type benzodiazepine binding sites in the hypophyseal-genital axis of rats. *Eur J Pharmacol* 1987;133:97-102.
 93. Bar-Ami S, Fares F, Gavish M. Effect of hypophysectomy and hormone treatment on the induction of peripheral-type benzodiazepine binding sites in female rat genital axis. *Horm Metab Res* 1989;21:106-7.
 94. Fares F, Bar-Ami S, Brandes JM, Gavish M. Changes in the density of peripheral benzodiazepine binding sites in genital organs of the female rat during oestrous cycle. *J Reprod Fertil* 1988;83: 619-25.
 95. Bar-Ami S, Fares F, Gavish M. Altered peripheral benzodiazepine receptor density in human granulosa-lutein cells in relation to follicular maturity. *Mol Cell Endocrinol* 1991;82:285-91.
 96. Gavish M, Weizman A, Okun F, Youdim MBH. Modulatory effects of thyroxine treatment on central and peripheral benzodiazepine receptors in the rat. *J Neurochem* 1986;47:1106-10.
 97. Christy N. The adrenal cortex in thyrotoxicosis. In: Braverman L, Utiger R, eds. *Werner and Ingbar's The Thyroid*. 6th ed. Philadelphia: JB Lippincott, 1991:806-20.
 98. Gamber S. Environmental effects and physiological variables. In: Braverman L, Utiger R, eds. *Werner and Ingbar's The Thyroid*. 6th ed. Philadelphia: JB Lippincott, 1991:347-57.
 99. Quirion R. High density of [³H]Ro 5-4864 peripheral benzodiazepine binding sites in the pineal gland. *Eur J Pharmacol* 1984;102:559-60.
 100. Weissman BA, Skolnick P, Klein DC. Regulation of peripheral binding sites for benzodiazepines in the pineal gland. *Pharmacol Biochem Behav* 1984;21:821-4.
 101. Matthew et al. Benzodiazepines: rat pinealocyte binding sites and augmentation of norepinephrine-stimulated *N*-acetyl transferase activity. *J Pharmacol Exp Ther* 1981;228:434-8.
 102. Calogero AE, Kamilaris TC, Bernardini R, Johnson EO, Chrousos GP, Gold PW. Effects of peripheral benzodiazepine receptor ligands on hypothalamic-pituitary-adrenal axis function in the rat. *J Pharmacol Exp Ther* 1990;253:729-37.
 103. Ferrarese C, Menmini T, Pecora N, et al. Diazepam binding inhibitor (DBI) increases after acute stress in rats. *Neuropharmacology* 1991;30:1445-52.
 104. Beaumont K, Healy DP, Fanestil DD. Autoradiographic localization of benzodiazepine receptors in rat kidney. *Am J Physiol* 1984;247:F718-24.
 105. Basile AS, Paul SM, Skolnick P. Adrenalectomy reduces the density of peripheral-type binding sites for benzodiazepine in rat kidney. *Eur J Pharmacol* 1985;110:149-50.
 106. Holmes PV, Drugan RC. Angiotensin II rapidly modulates the renal peripheral benzodiazepine receptor. *Eur J Pharmacol* 1992;226:189-90.
 107. Drugan RC, Basile AS, Crawley JN, Paul SM, Skolnick P. Inescapable shock reduces [³H]Ro 5-4864 binding to "peripheral-type" benzodiazepine receptors in the rat. *Pharmacol Biochem Behav* 1986;24:1673-7.
 108. Gavish M, Weizman A, Youdim MBH, Okun F. Regulation of central and peripheral benzodiazepine receptors in progesterone-treated rats. *Brain Res* 1987;409:386-90.
 109. Regan JW, Yamamura HI, Yamada S, Roeske WR. High-affinity renal [³H]flunitrazepam binding: characterization, localization, and alterations in hypertension. *Life Sci* 1981;28:991-8.
 110. Basile AS, Weissman BA, Skolnick P. Maximal electroshock increases the density of [³H]Ro 5-4864 binding to mouse cerebral cortex. *Brain Res Bull* 1987;19:1-7.
 111. Okun F, Weizman R, Katz Y, Bomzon A, Youdim MBH, Gavish M. Increase in central and peripheral benzodiazepine receptors following surgery. *Brain Res* 1988;458:31-6.
 112. Novas PT, Medina JH, Calvo D, De Robertis E. Increase in peripheral benzodiazepine binding sites in kidney and olfactory bulb in acutely stressed rats. *Eur J Pharmacol* 1987;135:243-6.
 113. Rāgo L, Kilvet RA, Harro J, Pold M. Central- and peripheral-type benzodiazepine receptors: similar regulation by stress and GABA receptor agonists. *Pharmacol Biochem Behav* 1989;32: 879-83.
 114. Rāgo L, Kilvet RA, Adojaan A, Harro J, Allikmets L. Stress-protective action of β -phenyl (GABA): involvement of central and peripheral type benzodiazepine binding sites. *Pharmacol Toxicol* 1990;66:41-4.
 115. Holmes PV, Stringer AP, Drugan RC. Impact of psychological dynamics of stress on the peripheral benzodiazepine receptor. *Pharmacol Biochem Behav* 1992;42:437-44.
 116. Karp L, Weizman A, Tyano S, Gavish M. Examination stress, platelet peripheral benzodiazepine binding sites, and hormone levels. *Life Sci* 1989;44:1077-82.

117. Drugan RC, Basile AS, Crawley JN, Paul SM, Skolnick P. Characterization of stress-induced alterations in [³H]Ro 5-4864 binding to peripheral benzodiazepine receptors in rat heart and kidney. *Pharmacol Biochem Behav* 1988;30:1015-20.
118. Drugan RC, Holmes PV, Stringer AP. Phenobarbital blocks the stress-induced decrease in [³H]Ro 5-4864 binding in rat kidney. *Brain Res* 1990;535:151-4.
119. Drugan RC, Holmes PV. Central and peripheral benzodiazepine receptors: involvement in an organism's response to physical and psychological stress. *Neurosci Biobehav Rev* 1991;15:277-98.
120. Deutsch SI, Mastropolo J, Hitri A. GABA-active steroids: endogenous modulators of GABA-gated chloride ion conductance. *Clin Neuropharmacol* 1992;15:352-64.
121. Drugan RC, Basile AS, Crawley JN, Paul SM, Skolnick P. Peripheral benzodiazepine binding sites in the Maudsley reactive rat: selective decrease confined to peripheral tissues. *Brain Res Bull* 1987;18:143-5.
122. Weizman A, Bidder M, Fares F, Gavish M. Food deprivation modulates γ -aminobutyric acid receptors and peripheral benzodiazepine binding sites in rats. *Brain Res* 1990;535:96-100.
123. Dar DE, Weizman A, Karp L, et al. Platelet peripheral benzodiazepine receptors in repeated stress. *Life Sci* 1991;48:341-6.